Splanchnic Bed Utilization of Enteral α-Ketoisocaproate in Humans

D.E. Matthews, R. Harkin, A. Battezzati, and D.J. Brillon

The branched-chain ketoacids (BCKAs) are used as dietary supplements to spare essential amino acid nitrogen, yet little is known about their absorption and utilization in the body. To study the fate of enterally delivered α-ketoisocaproate (KIC), seven healthy adults were infused in the postabsorptive state with [1-^{13}C]KIC and [phenyl-$^{2}H_5$]phenylalanine intravenously (NGI) and with [5,5,5-$^{2}H_3$]KIC by nasogastric tube (NG). After 3.5 hours, the routes of tracer infusion were switched for an additional 3.5 hours. Each subject received a second infusion study on a different day with the order of tracer infusion reversed. KIC and phenylalanine kinetics and first-pass uptake and disposal of the enteral tracer by the splanchnic bed were calculated from the tracer enrichments measured in plasma KIC, leucine, and phenylalanine and breath CO_2. Phenylalanine flux was 39.5 ± 1.2 μmol/kg/h during the IV infusion periods. KIC flux was 33.1 ± 1.8 and 30.4 ± 1.4 μmol/kg/h measured with ^{13}C- and $^{2}H_3$-KIC, respectively, and these values were significantly different. The fraction of enterally delivered tracer sequestered by the splanchnic bed on the first pass was 30.9% ± 2.0%, 30.0% ± 1.4%, and 30.7% ± 2.7% for ^{13}C-KIC, $^{2}H_3$-KIC, and $^{2}H_5$-phenylalanine, respectively. The fraction of infused ^{13}C-KIC tracer recovered as $^{13}CO_2$ was 27.1% ± 1.2% and 24.0% ± 0.9% during IV and NG infusion, respectively. From these data, the fraction of ng KIC tracer extracted and oxidized on the first pass was calculated to be 5.1% ± 1.1%. This fraction was greater than that previously reported for leucine extraction and oxidation (2%), but it was still only a small fraction of the overall extraction (5/30 = 16%). Because the only two fates of the KIC tracer extracted by the splanchnic bed are oxidation or transamination to leucine, the majority (84%) of the KIC tracer was extracted and converted to leucine. These results demonstrate that KIC delivered enterally to postabsorptive humans is rapidly extracted and predominantly converted to leucine by the splanchnic bed. This leucine appears to be available for use by both the splanchnic bed and the whole body.

ALPHA-KETOISOCAPROATE (KIC) is the transamination product of leucine. The transamination reaction is the first and reversible step in the leucine oxidative pathway. All three branched-chain amino acids (BCAAs), leucine, isoleucine, and valine, are transaminated by this common pathway to their respective branched-chain ketoacids (BCKAs), KIC, α-keto-β-methylvalerate, and α-ketoisovalerate, respectively.[1] The second step in BCAA metabolism is the decarboxylation of the BCKAs, also by a common dehydrogenase enzyme for the three BCKAs. The relative activities of these enzymes have been measured in a variety of tissues and animal species.[1-3] Transaminase activity is high in many tissues, but especially in muscle.[1,4] The dehydrogenase for decarboxylation of BCAAs to BCKAs is also active in muscle, making the BCAAs the only essential amino acids that are degraded outside of the liver.[5] However, in the rat there is considerable dehydrogenase activity in the liver,[2,6,7] suggesting that a considerable fraction of the BCKAs formed in muscle are transported to the liver for decarboxylation.[8] In humans, it is thought that most of the decarboxylation of BCAAs occurs in muscle.[1,3,5,9]

Studies with administration of a protein meal have demonstrated that most of the BCAAs pass through the splanchnic bed directly into systemic circulation and are taken up by muscle.[10,11] Using catheters in the splanchnic bed of dogs and labeled leucine tracers, Yu et al[12] demonstrated that only a small fraction of the leucine entering the gut or liver is extracted, and even less is oxidized. However, direct balance measurements of KIC were not made. Using labeled leucine tracers in humans (both in the postabsorptive state and in the fed state), we and others have evaluated the amount of enterally delivered leucine that is extracted on the first pass by the splanchnic bed.[13-16] The results of these studies indicate that 20% to 30% of the leucine delivered enterally is removed by the splanchnic bed on the first pass. We further determined for postabsorptive humans that less than 2% of the enteral leucine tracer was extracted by the splanchnic bed and oxidized.[16] However, from our measurements of the enrichment of plasma KIC during enteral leucine tracer infusion, we estimated that about 40% of the leucine extracted by the splanchnic bed was converted to KIC and released.[16] These results suggest that in human liver and/or gut, there is active transaminase for BCAAs but dehydrogenase activity for the BCAAs is low in the postabsorptive state.

The ketoacids of the BCAAs and other transaminating amino acids have been used as nitrogen-sparing substitutes for essential amino acids.[17] Although the use of ketoacids as nitrogen-sparing substitutes has been demonstrated,[9,17,18] little is known about the metabolism and utilization of ketoacids given enterally. Munoz and Walser[19] administered radiolabeled KIC and leucine enterally and measured the relative incorporation of the two tracers in albumin. They demonstrated that 63% of the KIC tracer relative to the leucine tracer was incorporated into albumin, suggesting that much of the enterally delivered KIC was transaminated by the splanchnic bed and used as leucine. Biolo and Tessari[20] administered leucine tracers by the intravenous (IV) and enteral routes and a ^{3}H-KIC tracer IV to four subjects. They measured leucine uptake and conversion to KIC by the splanchnic bed. Because they administered the KIC tracer only by the IV route, they did not determine enteral KIC extraction. They determined that relatively little of the enteral

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leucine extracted by the splanchnic bed was converted to KIC and released into the systemic circulation, and concluded that leucine-KIC transamination occurs largely in extrahepatic tissues.[20] This result is in contrast to our previous estimates of splanchnic bed metabolism of leucine.[13,16] Because transamination is rapid in both directions, their results[20] are also in contrast to the study by Munoz and Walser[19] indicating extensive splanchnic transamination of KIC.

Thus, the question remains as to how ketoacids such as KIC are handled by the splanchnic bed. To address this question, we measured the metabolism of KIC both in the whole body and in the splanchnic bed using stable isotopically labeled tracers of KIC administered IV and enterally to normal postabsorptive human subjects. A carbon-labeled tracer of KIC was used to directly measure first-pass uptake and oxidation of KIC by the splanchnic bed. The experimental design mirrors the design we used previously to study splanchnic bed utilization of leucine.[16]

SUBJECTS AND METHODS

Materials

Sodium α-[1-^{13}C]KIC (99% ^{13}C) was obtained from Mass Trace (Woburn, MA). Sodium α-[5,5,5-$^{2}H_3$]KIC (99% $^{2}H_3$) and sodium [^{13}C]bicarbonate were obtained from Cambridge Isotope Laboratories (Andover, MA). L-[phenyl-$^{2}H_5$]phenylalanine (93% $^{2}H_5$), DL-[$^{2}H_7$]leucine, and DL-[3,3-$^{2}H_2$]phenylalanine were obtained from the former MSD Isotopes (St Louis, MO). Sodium α-ketocaproate was purchased from Sigma Chemicals (St Louis, MO). Chemical and isotopic purities were determined by gas chromatography–mass spectrometry (GCMS). L-[phenyl-$^{2}H_5$]phenylalanine was tested for optical (absence of the D-stereoisomer) purity by GCMS. The compounds administered to human subjects were confirmed to be pyrogen-free prior to administration.

Prior to each infusion study, sterile solutions of the labeled amino acid tracers were prepared using aseptic technique. Accurately weighed amounts of each labeled compound were dissolved in known volumes of sterile saline and filtered through 0.22-μm Millipore (Bedford, MA) filters into 60-mL syringes for infusion. Solutions were prepared no more than 24 hours prior to use and were kept at 4°C.

Subjects

Seven healthy men (mean ± SD and range, respectively: age 36 ± 5 and 32 to 46 years; weight, 70 ± 11 and 58 to 91 kg; height, 175 ± 7 and 165 to 182 cm; and body mass index, 23 ± 4 and 18 to 28 kg/m^2) were studied at the New York Hospital-Cornell University Medical Center (NYH-CUMC) Clinical Research Center (CRC). A medical history, physical examination, and laboratory biochemical screening tests were used to confirm that each subject was free of clinical and laboratory evidence of disease. The subjects were instructed as to the purpose, benefits, and risks of the study and provided written consent in accordance with protocols approved by the NYH-CUMC Institutional Review Board and the CRC Scientific Advisory Committee.

Infusion Protocol

Each subject was admitted to the CRC (day 0) for a 4-day diet period (days 1 to 4) consisting of 38 kcal/kg/d and 1.2 g protein/kg/d. On the days prior to each infusion (days 1 and 3), the diet was given as a liquid formula (Ensure Plus; Ross Laboratories, Columbus, OH); otherwise, dietary intake was given as regular food. Infusions were performed on days 2 and 4 with subjects in the postabsorptive state having had nothing to eat or drink except water from 8 PM the previous evening until study completion at 3 PM the next day). On the evening prior to the infusions (days 1 and 3), an 8-Fr 109-cm weighted nasogastric (NG) tube (Corpak, Wheeling, IL) was placed. On the morning of the infusions, each subject was awakened at 7 AM and allowed to void. Catheters were placed in the antecubital fossa for IV infusion of labeled tracers and retrogradely in a dorsal hand vein of the opposite arm for blood sampling. The catheters were kept patent by slow infusion of sterile saline. The hand was warmed periodically in a heated-air box (air temperature 55°C) to produce "arterialized" venous blood.

Each subject received two 7-hour infusions and each infusion had two parts, period A (the first 3.5 hours) and period B (the second 3.5 hours). In study 1, [1-^{13}C]KIC and [$^{2}H_5$]phenylalanine were infused IV in period A while [$^{2}H_3$]KIC was infused simultaneously by the NG route. At the end of period A, the routes of infusion were reversed: [$^{2}H_3$]KIC was infused IV and [^{13}C]KIC and [$^{2}H_5$]phenylalanine were infused into the NG tube for another 3.5 hours. Study 2 was identical except that the order of infusion was the opposite of that for study 1: [$^{2}H_3$]KIC was infused IV and [^{13}C]KIC and [$^{2}H_5$]phenylalanine were infused by the NG route for period A and the routes of infusion were reversed for period B. The IV and NG infusion rates were the same for both studies 1 and 2, 1.8, 3.25, and 2.5 μmol/kg/h for [$^{2}H_5$]phenylalanine, [1-^{13}C]KIC, and [$^{2}H_3$]KIC, respectively. Just before the start of each infusion, a prime dose of [1-^{13}C]KIC (0.7 μmol/kg), [$^{2}H_3$]KIC (0.5 μmol/kg), [$^{2}H_5$]phenylalanine (1.6 μmol/kg), and sodium [1-^{13}C]bicarbonate (1.75 μmol/kg) was administered as an IV bolus. The volume infusion rate of the tracers was accurately determined by recording the starting and stopping times and weighing the infusion syringes at the beginning and end of each infusion.

During both studies, blood and breath samples were obtained prior to administration of the priming dose and at 15-minute intervals during the last 2 hours of each infusion period. Aliquots of blood were placed in tubes containing EDTA and stored on ice until the plasma was prepared by centrifugation at 4°C. A 0.5-mL aliquot of plasma was withdrawn and mixed with [$^{2}H_7$]leucine, [$^{2}H_2$]phenylalanine, and α-ketocaproate internal standards and frozen at −60°C. Breath samples were placed in 20-mL evacuated tubes and stored at room temperature until measurement of $^{13}CO_2$ in the expired air by isotope ratio mass spectrometry. At hourly intervals, CO_2 production was determined for 15-minute intervals using an indirect calorimeter with a flow-through hood (Deltatrak; Sensormedics, Yorba Linda, CA).

Analytical Methods

Plasma amino acid concentrations and enrichments were measured by negative chemical ionization GCMS (model 5988A; Hewlett-Packard, Palo Alto, CA), and plasma KIC concentration and enrichment were measured by electron-impact ionization GCMS (model 5970; Hewlett-Packard) as previously described.[21] Before measurement by GCMS, plasma amino acids and ketoacids from each sample were isolated and then derivatized separately. The amino acids were converted to the *N*-heptafluorobutyryl, *n*-propyl (HFBP) amino acid ester derivatives and the ketoacids to the *t*-butyldimethylsilyl-quinoxalinol derivatives.

For KIC, the [M-57]$^+$ ions at *m/z* 259, 260, and 262 were monitored for unlabeled KIC, [1-^{13}C]KIC, and [$^{2}H_3$]KIC, respectively.[22] The ketoacids that produce ions at *m/z* 259, ketocaproate, KIC, and α-keto-β-methylvalerate (the ketoacid of isoleucine), were all chromatographically resolved. The ketocaproate peak (*m/z* 259) eluted about 0.5 minutes after the KIC peak. The ratio of the KIC to ketocaproate peaks was used to determine the KIC concentration. Separate injections of the HFBP amino acid ester derivatives were made into the GCMS instrument for leucine and phenylalanine. Selected ion monitoring of the [M-HF]$^-$ ion was used. The ions at *m/z* 349, 350, 352, and 356 were monitored for unlabeled, [1-^{13}C], [$^{2}H_3$], and [$^{2}H_7$]leucine, and ions at *m/z* 383, 385, and 388 were monitored for unlabeled, [$^{2}H_2$], and [$^{2}H_5$]phenylalanine, respectively. For all GCMS measurements, the selected ion-monitoring peak areas of the measured ions were computed by the data system with background correction. In all cases, standards of

both known isotopic content (for ^{13}C and ^{2}H measurements) and concentration of unlabeled metabolite against the internal standard (for concentration measurements) were measured with each set of samples to calibrate the GCMS systems. The peak area ratios of the samples were transformed via the standard curves into ^{13}C and ^{2}H amino acid enrichment in mole percent excess (mpe) of tracer and into plasma concentration (by internal standard isotope dilution) values as previously described.[21,23] The ^{13}C content of CO_2 in exhaled air was measured by isotope ratio mass spectrometry using an in-house CO_2 standard of known ^{13}C and ^{18}O content. The measured exhaled-air $^{13}CO_2$ enrichments were expressed as atom percent excess ^{13}C (ape).

Calculations

The subscripts IV and NG refer to the enrichments during IV and NG infusion periods. The appearance rates into plasma (synonymous with "flux" or "turnover" in the steady state) of KIC and phenylalanine were calculated from the stochastic relationship,[24]

$$R_a = i\,[100/E_{p(IV)} - 1], \quad (1)$$

in micromoles per kilogram per hour, where $E_{p(IV)}$ is the amino acid tracer enrichment (mpe) in plasma at steady state and i is the infusion rate (micromoles per kilogram per hour) of labeled material (mass rate of tracer infusion times the fraction of tracer that is enriched, E). This expression accurately describes the appearance rate of the amino acid into the sampled compartment, plasma, regardless of the configuration of the model.

The NG-administered tracer will also enter systemic blood and be diluted. However, its entry rate into systemic blood is reduced by the fraction of tracer that is sequestered by the splanchnic bed on the first pass. Therefore, the enrichment in blood produced by the NG tracer will be lower than the enrichment produced by the IV tracer by the fraction of NG tracer sequestered by the splanchnic bed on the first pass.[16] The fraction of NG tracer sequestered on the first pass will be

$$f = 1 - E_{p(NG)}/E_{p(IV)}, \quad (2)$$

where $E_{p(NG)}$ and $E_{p(IV)}$ are the enrichment in plasma of the tracer infused by the NG and IV routes, respectively.

The rate of ^{13}C tracer oxidation (F_{13C}) was calculated by multiplying the rate of CO_2 production by the breath $^{13}CO_2$ enrichment for each subject. This rate was increased by 1/0.81, assuming that only 81% of the metabolic CO_2 produced is released as exhaled CO_2.[25] Dividing F_{13C} by the rate of tracer infusion (F_{13C}/i) yields the fraction of the infused tracer that was oxidized to CO_2. For the IV tracer, the fraction of tracer oxidized defines the systemic component of oxidation:

$$f_{ox(IV)} = F_{13C(IV)}/i. \quad (3)$$

For the NG tracer, the rate of $^{13}CO_2$ release ($F_{13C(NG)}$) will include both the tracer oxidized directly on the first pass ($f_{ox(NG)}$) and the tracer that escapes the splanchnic bed and is oxidized systemically after the first pass,

$$F_{13C(NG)}/i = f_{ox(NG)} + f_{ox(IV)} \cdot (1 - f), \quad (4)$$

where the first term reflects the fraction of NG tracer oxidized on the first pass and the second term reflects oxidation of the NG tracer that enters the systemic circulation.[16] The fraction of the NG ^{13}C tracer that is sequestered on the first pass and oxidized can be determined by rearranging Eq 4:

$$f_{ox(NG)} = F_{13C(NG)}/i - f_{ox(IV)} \cdot (1 - f). \quad (5)$$

Equation 5 represents one fate of the KIC NG tracer sequestered on the first pass: oxidation. The alternative fate is transamination of the sequestered KIC into leucine, which can be released into the systemic circulation.

KIC oxidation (C, micromoles per kilogram per hour) was calculated during the IV tracer infusion as previously described,[24]

$$C = F_{13C(IV)}\,[1/E_{p(IV)} - 1/E] \cdot 100, \quad (6)$$

using as the precursor enrichment ($E_{p(IV)}$) either plasma [1-^{13}C]KIC (primary pool enrichment) or [1-^{13}C]leucine (reciprocal pool enrichment).[26]

Statistics

Data are presented as the mean ± 1 SE. During the tracer infusions, steady state was defined as an insignificant correlation of a parameter ($P > .05$) with time using standard linear regression. A linear regression analysis was performed for each enrichment and concentration parameter versus time for each subject's infusion to calculate the rate of change of each parameter with time. Steady state for each parameter was defined as a mean slope of all infusions not significantly different from zero ($P > .05$). The testing was performed separately for periods A and B. Because the KIC ^{13}C and ^{2}H tracers should produce identical results with respect to the time course of enrichment, [1-^{13}C]KIC and [$^{2}H_3$]KIC enrichments were tested together for common infusion routes (eg, iv first, which was study 1 period A for the ^{13}C tracer and study 2 period B for the ^{2}H tracer). Testing for significant differences between parameters was performed by repeated-measures ANOVA (RMANOVA) using BMDP2V (BMDP Statistical Software, Los Angeles, CA). Testing assessed differences between the response of the ^{13}C- and $^{2}H_3$-KIC tracers, differences between the order of tracer infusion (studies 1 and 2), and differences between periods A and B. In some cases, only one factor was tested, and testing reduced to a paired *t* test using a pooled error term.

RESULTS

The plasma measurements of the amino and keto acid concentration and tracer enrichment for the two infusions in the seven subjects are shown in Table 1. Each subject received two infusions, each divided into two halves. The route of tracer infusion (IV *v* NG) was switched between the two halves of each infusion. The order of infusion (ie, IV-NG or NG-IV) was the only difference between study 1 and study 2. The order of studies 1 and 2 was randomized among subjects. The data presented in Table 1 are mean data for the first and second halves (parts A and B) of each infusion study. There were no significant differences between the two studies or between the first and second halves of the studies in terms of the amino acid concentration. Linear regression analysis of the KIC concentration over the entire infusion period defined a small significant ($P < .001$) increase of 1.5 ± 0.3 μmol/L/h (3%/h) during the infusion period. This increase is assumed to be associated with progressive fasting during the infusion.

The time course of the plasma enrichments are presented in Figs 1 to 3, and the mean enrichments for the last 1.5 hours of each infusion period are presented for all tracers and studies in Table 1. The plasma tracer enrichments were at isotopic steady state (as defined by a nonsignificant change in enrichment with time during the last 1.5 hours of each infusion period) for each tracer and infusion study. As expected, the plateau for the NG infusion periods tended to have more variability from time point to time point than the corresponding IV infusion period enrichments. The enrichments of all three infused tracers ([1-^{13}C]KIC, [$^{2}H_3$]KIC, and [$^{2}H_5$]phenylalanine) were significantly lower during infusion of the tracers by the NG route versus the IV route (Table 1 and Figs 1 to 3), indicating partial

Table 1. Plasma Enrichment and Concentration of KIC, Leucine, and Phenylalanine

Parameter	Study 1		Study 2	
	A	B	A	B
Plasma amino acid concentration (μmol/L)				
KIC	44.0 ± 4.9	49.6 ± 4.3	41.4 ± 2.9	47.4 ± 3.7
Leucine	122.5 ± 7.3	126.6 ± 9.8	122.6 ± 6.2	136.9 ± 14.7
Phenylalanine	61.1 ± 1.5	57.1 ± 1.9	60.0 ± 1.5	64.3 ± 3.0
Plasma enrichment (mpe)				
[1-^{13}C]KIC	9.81 ± 0.71	6.43 ± 0.48	6.38 ± 0.43	8.64 ± 0.51
[1-^{13}C]leucine	2.34 ± 0.14	2.61 ± 0.14	2.21 ± 0.17	2.6 ± 0.17
[$^{2}H_3$]KIC	7.61 ± 0.50	5.47 ± 0.36	5.40 ± 0.30	7.94 ± 0.37
[$^{2}H_3$]leucine	1.97 ± 0.10	1.73 ± 0.10	1.93 ± 0.16	1.70 ± 0.14
[$^{2}H_5$]phenylalanine	4.40 ± 0.13	3.19 ± 0.19	2.86 ± 0.17	4.37 ± 0.21
Leucine/KIC enrichment ratio (%)				
^{13}C-leucine/^{13}C-KIC	24.7 ± 2.2	43.2 ± 3.3	36.9 ± 3.8	30.5 ± 2.8
$^{2}H_3$-leucine/$^{2}H_3$-KIC	33.0 ± 1.5	27.3 ± 1.2	21.7 ± 1.6	35.8 ± 2.3

NOTE. Data are the mean enrichment for each subject for the last 1.5 hours of each period averaged together, presented as the mean ± SE for 7 subjects. Enrichment data are missing for $^{2}H_5$-phenylalanine for one subject; n = 6 for this parameter. Plasma concentrations were not different between studies or periods for leucine or phenylalanine. Plasma KIC concentration was not different between studies, but was higher ($P < .05$, RMANOVA) in period B *v* period A. The higher enrichment values for [1-^{13}C]KIC tracer are a function of the faster infusion rate *v* [$^{2}H_3$]KIC. RMANOVA for differences between ^{13}C and ^{2}H tracers for both KIC and leucine was performed after normalizing the enrichment for the tracer infusion rate (dividing enrichment by infusion rate of tracer). The normalized ^{2}H- and ^{13}C-KIC enrichments were significantly lower during NG *v* IV infusion ($P < .001$). There was no difference in KIC enrichments between studies 1 and 2 (time effect, IV first *v* NG first). The normalized ^{2}H-KIC enrichments were significantly higher than the ^{13}C-KIC enrichments ($P < .01$). The normalized ^{2}H- and ^{13}C-leucine enrichments were not different between IV and NG infusions or between ^{2}H and ^{13}C tracers. There was a significant difference in leucine enrichments between studies 1 and 2 (time effect, IV first *v* NG first, $P < .001$). Phenylalanine enrichments were significantly lower during NG *v* IV infusion ($P < .001$); there was no difference in enrichments between studies 1 and 2 (RMANOVA). The ratio of leucine/KIC enrichments was significantly higher for both tracers when the tracers were infused by the NG route *v* the IV route ($P < .001$), and there was a significant difference in the enrichment ratio between studies 1 and 2 (time effect, IV first *v* NG first, $P < .001$). There was no effect of tracer (^{2}H *v* ^{13}C).

removal of the NG infused tracers on the first pass by the splanchnic bed. ^{13}C-KIC enrichment values were higher than the corresponding ^{2}H-KIC enrichment values because the ^{13}C-KIC tracer was infused at a faster rate than the ^{2}H-KIC tracer to obtain sufficient enrichment in exhaled $^{13}CO_2$. However, after normalizing the KIC ^{13}C and ^{2}H enrichments for the infusion rate, a significant difference between the two was still noted.

The tracer enrichment in leucine, which was derived from the KIC tracer transamination to leucine, was significantly lower ($P < .001$) than the KIC plasma enrichment for both the ^{13}C and ^{2}H tracers. The leucine enrichment was not different between the IV and NG infusions. The enrichment of leucine relative to KIC (the ratio of leucine enrichment divided by KIC enrichment) increased significantly when switching from infusion of KIC tracer by the IV route to the NG route (Table 1), indicating that the splanchnic bed converted some of the ng-delivered KIC tracer directly to leucine. There was no difference in the leucine/KIC enrichment ratio between the ^{13}C- and $^{2}H_3$-KIC tracer infusions.

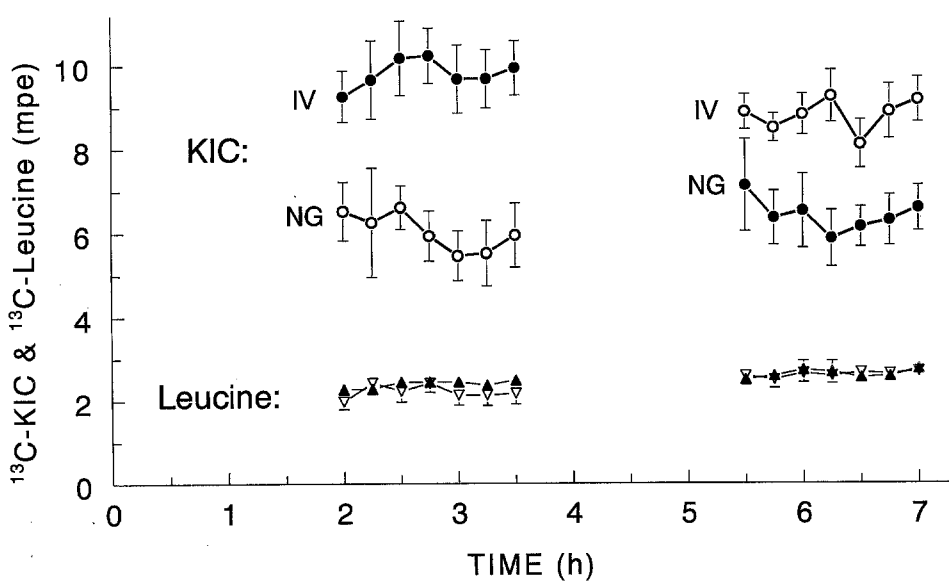

Fig 1. Time course of plasma enrichments for KIC and leucine during infusion of [1-^{13}C]KIC. In study 1, enrichment data (mpe) are for [1-^{13}C]KIC (●) and [1-^{13}C]leucine (▲), where [1-^{13}C]KIC was infused by the IV route for the first 3.5 hours and by the NG route for the second 3.5 hours. In study 2, enrichment data are for [1-^{13}C]KIC (○) and [1-^{13}C]leucine (▽), where [1-^{13}C]KIC was infused by the NG route first and by the IV route last. Data are the mean ± 1 SE error bars. Error bars smaller than the symbols are not shown.

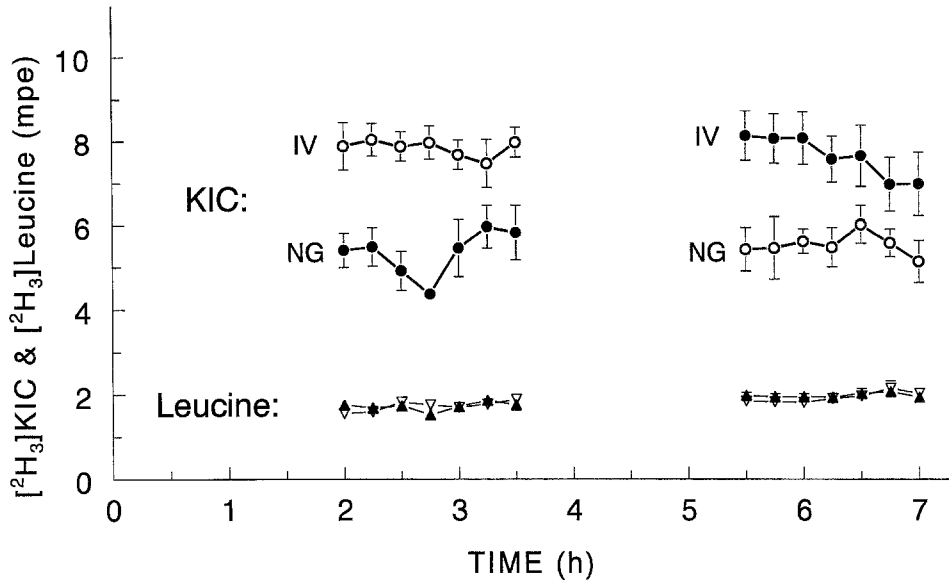

Fig 2. Time course of plasma enrichments for KIC and leucine during infusion of [$^2H_3$]KIC. In study 1, enrichment data (mpe) are for [$^2H_3$]KIC (●) and [$^2H_3$]leucine (▲), where [$^2H_3$]KIC was infused by the NG route for the first 3.5 hours and by the IV route for the second 3.5 hours. In study 2, enrichment data are for [$^2H_3$]KIC (○) and [$^2H_3$]leucine (▽), where [$^2H_3$]KIC was infused by the IV route first and by the NG route last. Data are the mean ± 1 SE error bars. Error bars smaller than the symbols are not shown.

The KIC and phenylalanine appearance rates were calculated from the plateau enrichments during the IV periods and are presented in Table 2. There was no statistical difference in the rate of appearance (R_a) measured between studies (period A, IV first, *v* period B, IV second) for phenylalanine or KIC. However the KIC R_a was significantly lower ($P < .05$) when measured with the $^2H_3$-KIC versus ^{13}C-KIC tracer, indicating that the two tracers yielded slightly different measures of KIC kinetics.

The fraction of the NG tracer sequestered on the first pass was calculated for the three different tracers by comparing IV and NG plateau enrichments from the same infusion day for each subject. These values are shown in Table 2 for [1-^{13}C]KIC, [$^2H_3$]KIC, and [$^2H_5$]phenylalanine (f = 30.9% ± 2.1%, 30.0% ± 1.4%, and 30.7% ± 2.7%, respectively). The value for f was not different between the ^{13}C- and $^2H_3$-KIC tracers, nor was it different between study 1 (IV first) and study 2 (NG first). The amount of tracer sequestered on the first pass was significantly ($P < .001$) greater than zero for both KIC and phenylalanine.

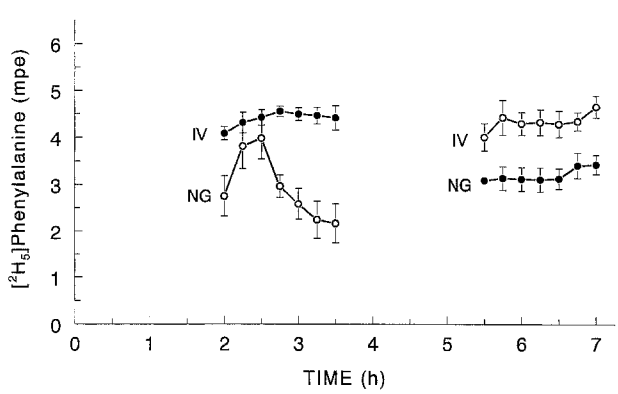

Fig 3. Time course of plasma enrichments for phenylalanine during infusion of [ring-$^2H_5$]phenylalanine. In study 1 (●), [$^2H_5$]phenylalanine enrichment data (mpe) are for tracer infused by the IV route for the first 3.5 hours and by the NG route for the second 3.5 hours. In study 2 (○), [$^2H_5$]phenylalanine enrichment data are for tracer infused by the NG route first and by the IV route last. Data are the mean ± 1 SE error bars. Error bars smaller than the symbols are not shown.

The time course of ^{13}C-KIC oxidation expressed as the fraction of infused ^{13}C tracer recovered as $^{13}CO_2$ (rate of $^{13}CO_2$ excretion divided by KIC ^{13}C infusion rate) is shown in Fig 4. The rate of $^{13}CO_2$ release was in steady state for the last 1.5 hours of each tracer infusion period. The data (rate of CO_2 production and breath $^{13}CO_2$ enrichment) used to calculate $^{13}CO_2$ tracer excretion are presented in Table 3. The CO_2 production rate did not differ between study 1 and study 2 for individual subjects. KIC oxidation was calculated from the rate of $^{13}CO_2$ excretion using both plasma [1-^{13}C]KIC and [1-^{13}C]leucine (reciprocal pool) as the precursor enrichments. Because the plasma leucine ^{13}C was significantly lower than the plasma KIC ^{13}C enrichment, the oxidation determined using leucine ^{13}C was significantly higher than the oxidation calculated using plasma KIC ^{13}C directly. Oxidation was calculated only for the IV infusion of ^{13}C tracer, as some tracer will be extracted and oxidized directly on the first pass during NG infusion.

As expected, $^{13}CO_2$ enrichment and the rate of $^{13}CO_2$ excretion were significantly lower during ng infusion compared with iv infusion (Table 3). The amount of NG-infused ^{13}C-KIC tracer that was oxidized directly on the first pass was calculated from the fractional oxidation of ^{13}C-KIC tracer between the IV and NG routes for each subject and each infusion (Eq 5). The amount of enterally delivered tracer oxidized directly on the first pass was 5.7% ± 1.7% and 4.4% ± 1.5% for studies 1 and 2, respectively (Table 3). There was no significant difference between studies (order of tracer delivery). The mean fraction of the NG-delivered KIC tracer oxidized on the first pass for both studies was 5.1% ± 1.1% and was significantly greater than zero.

Thus, 30% of NG-delivered KIC was extracted on the first pass by the splanchnic bed, and 5/30, or 16% ± 4% (mean of individual infusions), of this material was oxidized directly in the splanchnic bed. The remaining 84% of the NG tracer that was sequestered on the first pass was converted to leucine—the only alternative fate to KIC oxidation.

Table 2. KIC and Phenylalanine R_a and First-Pass Extraction by the Splanchnic Bed

Parameter	Study 1 A	Study 1 B	Study 2 A	Study 2 B	Mean of Both Studies
Ra (μmol/kg/h) determined by:					
[^{13}C]KIC	30.9 ± 2.4			35.3 ± 2.6	33.1 ± 1.8
[$^{2}H_3$]KIC		31.3 ± 2.4	29.5 ± 1.5		30.4 ± 1.4
[$^{2}H_5$]phenylalanine	39.2 ± 1.4			39.8 ± 2.2	39.5 ± 1.2
Fraction (f) of NG tracer extracted on first pass (%) determined by:					
[^{13}C]KIC	34.1 ± 3.2		27.8 ± 2.0		30.9 ± 2.0
[$^{2}H_3$]KIC	27.9 ± 2.2		32.1 ± 1.3		30.0 ± 1.4
[$^{2}H_5$]phenylalanine	27.3 ± 4.2		34.2 ± 3.2		30.7 ± 2.7

NOTE. R_a was calculated from the tracer enrichments from the iv infusion periods as noted. First-pass extraction was calculated from IV and NG data from the same infusion study on the same day (ie, periods A and B for each infusion). n = 7 for KIC and n = 6 for phenylalanine. There was no significant difference for either KIC R_a (RMANOVA) or phenylalanine R_a (paired *t* test) between periods A and B (order of infusion as IV or NG first). However, the KIC R_a was lower when measured using $^{2}H_3$- *v* ^{13}C-KIC tracer ($P < .05$, RMANOVA). First-pass extraction was not different between ^{2}H- and ^{13}C-KIC tracers, nor was it different with respect to infusion order (IV or NG first). There was no difference in f with respect to infusion order for phenylalanine (paired *t* test).

DISCUSSION

Ketoacids have been used as nitrogen-sparing dietary substitutes for essential amino acids that transaminate (eg, BCAAs) in humans who must be restricted in nitrogen intake (eg, patients with renal disease).[9,17,18,27] However, the basis for the use of dietary ketoacids in humans is largely from empirical studies of nitrogen-sparing rather than from data of ketoacid absorption and utilization in humans. Investigations have been performed in animal models where labeled KIC and leucine have been administered by both IV and oral routes to rats.[6,27,28] These studies demonstrated that enteral KIC was about 40% as effective as leucine in terms of the conversion and utilization as leucine for protein synthesis.[27,28] The assumption was that the other 60% of the KIC was oxidized.[6] When Weber et al[29] infused KIC directly into surgically ligated dog jejunum, they found that only 8% appeared in blood and one third was degraded by the gut wall. When Abumrad et al[30] administered a large oral load of KIC to dogs with catheters across different organs (gut, liver, and kidney), they found that about 60% was removed by the gut and liver on the first pass and about half of the sequestered KIC was converted to leucine. Oxidation was not studied. The results of these studies suggest that less than half of the administered KIC is converted by the gut to leucine.

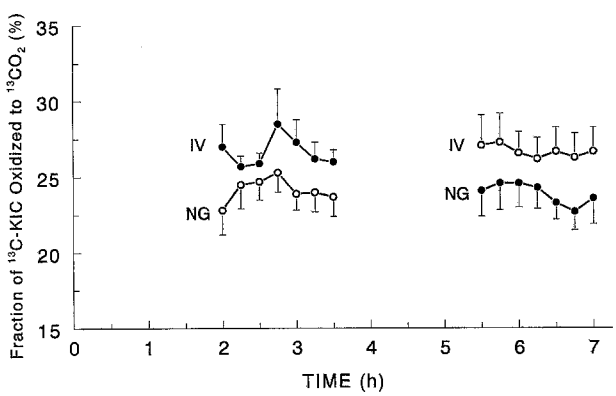

Fig 4. Time course of $^{13}CO_2$ excretion in breath during ^{13}C-KIC tracer infusion. Data are expressed as the rate of exhaled $^{13}CO_2$ divided by the rate of infused KIC ^{13}C (F_{13C}/i). (●) Study 1; (○) study 2. Data are the mean ± 1 SE error bars.

Studies of the fate of enteral ketoacids in the splanchnic bed in humans are sparse. Munoz and Walser[19] measured the incorporation of enterally delivered [1-^{14}C]KIC and ^{3}H-leucine into secreted hepatic proteins. They found 63% as much ^{14}C label from KIC in protein leucine as ^{3}H-leucine label. These results are similar to the previous results in animals on the utilization of enteral KIC as leucine, but they do not address first-pass metabolism or oxidative metabolism of enteral KIC and its related kinetics.

We performed the present study in an effort to fill the void in the knowledge of the splanchnic metabolism of KIC in humans. We used a similar infusion protocol that we previously used for measuring splanchnic uptake and extraction of leucine and phenylalanine in normal subjects.[16] The design uses a crossover approach whereby tracer is infused by one route (IV or NG) an then changed to the opposite route for the second half of the infusion. In contrast to the former study,[16] the same (rather than different) subjects received both the IV-NG and NG-IV infusions on separate occasions. Because two tracers of KIC were available (as was also the case for leucine in the prior study), we administered two tracers of KIC simultaneously but by different routes.

Although there was a small significant increase in the plasma KIC concentration over time during the tracer infusion, the concentration of leucine and the tracer enrichments in blood of KIC, leucine, and phenylalanine and in breath CO_2 were in steady state during both the IV and NG portions of the infusion. The same tracer infusion rate was used for the IV and NG portions for each tracer. Thus, the lower plasma tracer enrichments during NG infusion compared with IV infusion for both the KIC and phenylalanine tracers (Figs 1 to 3 and Table 2) directly define removal of the tracers on the first pass by the splanchnic bed when delivered enterally.

The amount of phenylalanine extracted in this study (f = 30.7% ± 2.7%) was not different from the amount we determined previously using the same study design (f = 26.4% ± 3.5%) when leucine tracers were coadministered.[16] The phenylalanine flux also was not different between this study (R_a = 39.5 ± 1.2 μmol/kg/h) and the former study (R_a = 39.1 ± 2.2 μmol/kg/h).[16] The similarities in phenylalanine kinetics between studies suggest that we can compare the KIC results of this study with the leucine results of the former study. The

Table 3. Carbon Dioxide Production and ^{13}C Enrichment and Excretion of Exhaled ^{13}C-KIC Tracer

	CO_2 Production (mmol/kg/h)	Breath $^{13}CO_2$ Enrichment (mpe × 1,000)	$^{13}CO_2$ Excretion (F_{13C}, μmol/kg/h)	KIC Oxidation (μmol/kg/h) KIC Precursor	KIC Oxidation (μmol/kg/h) Leucine Precursor	Fraction of Tracer Oxidized to CO_2 (F_{13C}/i, %)	Fraction of ng Tracer Oxidized on First Pass ($f_{ox(ng)}$, %)
Study 1	7.45 ± 0.50						
IV A		9.70 ± 0.39	0.888 ± 0.063	8.6 ± 1.1	38.6 ± 4.8	27.5 ± 1.8	
NG B		8.55 ± 0.72	0.768 ± 0.044			23.9 ± 1.4	5.7 ± 1.7
Study 2	7.26 ± 0.44						
NG A		8.82 ± 0.49	0.779 ± 0.035			24.1 ± 1.2	
IV B		9.81 ± 0.78	0.863 ± 0.052	9.2 ± 0.9	33.6 ± 3.5	26.7 ± 1.6	4.4 ± 1.5
Mean ± SE of both studies 1 and 2							
IV		8.68 ± 0.42	0.774 ± 0.027	8.9 ± 0.7	36.1 ± 2.9	27.1 ± 1.2	
NG		9.76 ± 0.42	0.876 ± 0.039			24.0 ± 0.9	5.1 ± 1.1

NOTE. F_{13C} is the product of CO_2 production times breath $^{13}CO_2$ enrichment, adjusted for body bicarbonate retention. KIC oxidation was calculated from F_{13C} using both plasma [1-^{13}C]KIC and [1-^{13}C]leucine as the precursor enrichment, as indicated. All calculations were performed for individual subjects, and then the mean ± SE values reported here were calculated. There was no significant difference in CO_2 production between studies 1 and 2 (paired *t* test). Because the leucine and KIC ^{13}C enrichments were significantly different (Table 1), KIC oxidation was significantly ($P < .0001$) different when calculated using leucine *v* KIC as a precursor. There was no difference in KIC oxidation between studies 1 and 2 using either KIC or leucine as the precursor enrichment (paired *t* tests). The fraction of tracer oxidized to CO_2 (F_{13C}/i) was significantly lower (RMANOVA, $P < .05$) when delivered by the NG *v* IV route. There was no effect ($P > .05$, RMANOVA) of infusion order (IV first or second) on F_{13C}/i. There was no significant difference in $f_{ox(ng)}$ between studies 1 and 2 (paired *t* test). $f_{ox(ng)}$ was significantly ($P < .001$) greater than zero.

amount of KIC extracted by the splanchnic bed on the first pass was 30.4% ± 1.7%, compared with 19% extraction of enteral leucine on the first pass.[16] Thus, 60% more KIC was extracted by the splanchnic bed compared with leucine. The question then becomes what is the fate of this sequestered KIC—is it for oxidation, or is the KIC converted to leucine, becoming available for use as an amino acid?

We measured the fraction of ^{13}C-KIC tracer oxidized when infused by the IV and NG routes. When KIC was infused IV, 27.1% ± 1.2% was oxidized; the remaining 73% was converted to leucine, the only other fate of KIC metabolism other than oxidation. When KIC was administered enterally, the proportions did not change much: 24.0% ± 0.9% was oxidized and 76% was converted to leucine. The question is how much NG tracer oxidation occurred in the splanchnic bed. The NG tracer oxidation includes both the tracer sequestered on the first pass and oxidized and the tracer that entered the systemic circulation and was subsequently oxidized. Using Eq 6 to dissect these components, we determined that only 5.1% ± 1.1% of the enteral ^{13}C-KIC tracer was oxidized on the first pass by the splanchnic bed. This amount of tracer oxidation represents 16% ± 4% of the KIC tracer extracted by the splanchnic bed. Thus, 84% ± 4% of the extracted KIC was converted to leucine.

These results demonstrate that in postabsorptive humans, the splanchnic bed extracts KIC not for oxidation but primarily for transamination to leucine. Suryawan et al[3] measured BCAA transaminase and BCKA dehydrogenase activity in a variety of human, rat, and primate tissues. They determined a high ratio of transaminase activity to dehydrogenase activity in primates, indicating that the gastrointestinal tract and liver play an important role in whole-body transamination of BCAAs and BCKAs. Taniguchi et al[31] found measurable transaminase in human liver and determined that the BCKA dehydrogenase activity was about 1% of the activity found in rat liver. Thus, the enzyme activity data in primates and man support our in vivo data that the splanchnic bed has significant activity for transamination of KIC to leucine and vice versa while expressing limited activity for oxidizing KIC.

As discussed previously for leucine,[16] the relative enrichment in plasma of the transaminated product formed from the tracer can be measured and compared for the IV and NG tracer infusions to indicate the relative contribution of the splanchnic bed to whole-body transamination. In the former report, we demonstrated that the plasma tracer enrichment ratio of KIC/leucine increased from 78% during IV infusion of leucine tracer to 93% during ng infusion, indicating direct conversion of ng leucine tracer to KIC by the splanchnic bed.[16] In the present study using a KIC tracer, we found that the plasma enrichment ratio of leucine/KIC increased from 26.0% ± 1.6% during IV infusion to 37.2% ± 2.1% during NG infusion (Table 1 data for the ^{13}C and ^{2}H tracers combined for studies 1 and 2). The leucine/KIC enrichment ratio was higher during NG infusion compared with IV infusion (0.37/0.26 = 1.42) and considerably higher than the KIC/leucine enrichment ratio when the leucine tracer was infused by the NG and IV routes (0.93/0.78 = 1.20).[16] The higher ratio for KIC conversion versus leucine conversion (1.42 *v* 1.20) indicates that the splanchnic bed will convert enteral KIC to leucine as efficiently, if not more efficiently, as it converts leucine to KIC.

There are few reports of KIC kinetics in the literature for humans. We determined that the rate of appearance of KIC into plasma was 33.1 ± 1.8 and 30.4 ± 1.4 μmol/kg/h using [1-^{13}C]KIC and [$^{2}H_3$]KIC tracers, respectively. There was a small but significant difference between the two tracer measurements (33.1/30.4 − 1 = 9%). The GCMS measurements of the two tracers were completed with simultaneous measurement of standard samples of the same materials that were infused. Any error or uncertainty in the assumption of either KIC tracer's purity would affect the standard sample and plasma sample measurements equally and cancel from the enrichment calculations. Thus, the effective difference measured between the two tracers is not likely related to any problem in tracer purity. The

difference in flux rates between the two tracers appears real. We speculate that there may be an isotope effect associated with the metabolism of the deuterated tracer. Because there was no difference between ^{13}C and ^{2}H tracers in terms of intracellular metabolism (ie, measurement of first-pass extraction or transamination to leucine), we assume that the difference found between the two KIC tracers is related to differences in the tracers in plasma. One possibility of an isotope effect could be at the level of KIC binding to plasma proteins[32] in that the deuterated KIC may bind more tightly, be retained in plasma longer, and therefore demonstrate a slower flux. However, this conclusion is speculative. What appears to be true is that the two tracers are not equivalent and cannot be used interchangeably.

Our measurement of KIC flux is similar to other reports in the literature. Schwenk et al[26] infused individuals with [1-^{14}C]- and ^{3}H-KIC and measured a KIC R_a of 34.8 ± 1.2 and 37.2 ± 3.0 μmol/kg/h, respectively. Their study showed no significant effect of the tracer on the flux, but different groups of subjects were used for the different tracers, reducing the statistical power.[26] In addition, the tritium isotopes were not concentrated in a single methyl group in the KIC tracer and would not be subject to the same isotope effect as might potentially occur with [5,5,5-$^{2}H_3$]KIC. Jensen et al[33] measured a KIC flux of 33.0 ± 1.8 μmol/kg/h using a [$^{2}H_3$]KIC tracer. Tessari et al[34] determined a faster KIC R_a of 56.4 ± 7.2 μmol/kg/h using a [1-^{14}C]KIC tracer in the normal subjects in this study. This latter fast rate of KIC flux cannot be readily explained, but illustrates the variability of results reported.

KIC oxidation was measured in this study and used to determine fractional oxidation. The whole-body rate of KIC oxidation was also calculated for the IV [1-^{13}C]KIC tracer infusion periods. KIC oxidation was 8.9 ± 0.7 μmol/kg/h using plasma KIC ^{13}C enrichment as the precursor. Tessari et al have two published reports of KIC oxidation in humans using plasma [1-^{14}C]KIC as precursor. KIC oxidation was 10.2 ± 1.2 μmol/kg/h in the first study[34] and 9.6 ± 1.8 μmol/kg/h in the second.[35] These values for KIC oxidation in normal postabsorptive subjects are not different from the values measured in the present study.

By simultaneous infusion of KIC tracers via the IV and NG routes, we have been able to demonstrate not only the relative amount of KIC tracer extracted by the splanchnic bed but also the fate of the KIC uptake—oxidation or transamination to leucine. On a whole-body basis, about one fourth of the administered KIC tracer was oxidized, with the other three fourths converted to leucine. The proportion of enteral KIC tracer oxidized by the splanchnic bed was similar to systemic kinetics, but this result reflects primarily the systemic portion of the enteral tracer metabolism. Our method allows us to dissect this measurement to define the splanchnic bed–specific component. The splanchnic bed removed one third of the NG-infused KIC tracer on the first pass, of which only 16% was oxidized. This result demonstrates direct oxidation of KIC by the splanchnic bed, but it also demonstrates that the fraction of KIC oxidation by the splanchnic bed on the first pass was significantly lower than systemic fractional oxidation. The conclusion from this latter observation is that the splanchnic bed is not the major site of KIC oxidation in the body. However, the splanchnic bed did extract KIC in amounts much greater than its rate of KIC oxidation. Thus, this result and the finding of a higher plasma leucine enrichment during NG versus IV infusion of KIC tracers demonstrate that the splanchnic bed is a significant site of transamination of KIC to leucine.

Although the results of this study are relevant only to postabsorptive humans, they form a basis from which results wherein ketoacids are given as a load may be extrapolated. The results for administration of a KIC load to dogs[30] are not very different from our results for postabsorptive humans. Further studies are needed to confirm this hypothesis.

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